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Human Immunodeficiency Virus Type 2 Envelope Glycoprotein Binds to CD8 as Well as to CD4 Molecules on Human T Cells

HIROSHI KANEKO,[1] LIAN-PIN NEOH,[1] NANAKO TAKEDA,[1] HIROAKI AKIMOTO,[1] TAKASHI HISHIKAWA,[1] HIROSHI HASHIMOTO,[1] SHUN-ICHI HIROSE,[2] SACHIKO KARAKI,[3] MASAFUMI TAKIGUCHI,[3] HIROMITSU NAKAUCHI,[4] YUTARO KANEKO,[5] NAOKI YAMAMOTO,[6] AND IWAO SEKIGAWA[2]*

Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine,[1] and Department of Microbiology, Tokyo Medical and Dental University School of Medicine,[6] Bunkyo-ku, Tokyo 113, Department of Medicine, Juntendo University Izu-Nagaoka Hospital, Tagata-gun, Shizuoka 410-22,[2] Department of Tumor Biology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108,[3] Department of Immunology, Institute of Basic Medical Sciences and Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Tsukuba Science-City, Ibaraki 305,[4] and Ajinomoto Co., Inc., Chuo-ku, Tokyo 104,[5] Japan

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We report here that human immunodeficiency virus type 2 (HIV-2) envelope glycoprotein (gp105), but not HIV-1 gp120, can bind to CD8 molecules as well as to CD4 molecules on human T cells. This phenomenon may lead to differences in the life cycles of HIV-1 and HIV-2, and it may be related to the differences in disease manifestations of HIV-1 and HIV-2 infection, including longer survival of HIV-2-infected patients.

Human immunodeficiency virus type 2 (HIV-2), the second AIDS-associated human retrovirus, was isolated and found to be quite common in west Africa (17, 25), and its molecular structure is more closely related to simian immunodeficiency virus (SIV), a nonhuman primate retrovirus, than to the first described human AIDS virus (HIV-1) (5). Due to the more restricted geographic spread, reduced transmission rate, and lower disease potential of this virus (2, 17, 25, 26), both basic and clinical research on HIV-2 have been less extensive than research on HIV-1, which is more widespread. Because HIV-2 differs from HIV-1 with respect to its biological and molecular behaviors, as well as its genomic structure, it may not be appropriate to apply the information gained from one virus directly to the other. Here we report that the envelope glycoprotein of HIV-2, but not that of HIV-1, can bind not only to CD4 molecules, but also to CD8 molecules, on human T cells.

Unlabeled and fluorescein isothiocyanate (FITC)-labeled recombinant gp105 (HIV-2_{ROD}) and HIV-1 envelope glycoprotein gp120 (HIV-1_{IIIB}) obtained from a baculovirus expression system (Intracel Co., Issaquah, Wash.) were used. Cultures (10^6 cells/1 ml per well) were performed in a 5% CO_2 incubator at 37°C in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U of penicillin/ml, 100 μg of streptomycin/ml, and 2 mM L-glutamine (Gibco Laboratories, Grand Island, N.Y.). To observe the binding of HIV envelope protein, we used cultures grown for 24 h at 37°C, based on the result of time course experiments. Although binding effects were observed even in shorter-term cultures grown at 37 and at 4°C, the former culture conditions were thought to be best for our binding assay system. With regard to gp120, in longer-term cultures (e.g., 48 to 72 h), down-modulation of CD4 on cell surfaces was observed, probably due to internalization of complexes of HIV envelope protein and CD4 molecules into cells (20), as previously reported (8, 19). Therefore, we thought that longer-term cultures were not adequate for the purpose of binding experiments. $CD4^+$ and $CD8^+$ T cells were purified from normal human peripheral blood mononuclear cells (PBMC) by negative selection with an immunoadsorption column (Cellect; Biotex Laboratories, Inc., Edmonton, Alberta, Canada), as described previously (16). Macrophages adherent to petri dishes (Corning Glass Works, Corning, N.Y.) were collected by incubating PBMC for 120 min at 37°C, as described previously (36), and then were removed before cells were passed through the column. The $CD4^+$ and $CD8^+$ T cells prepared by these procedures were recognized by a Leu 3a antibody and a Leu 2a antibody (Becton Dickinson, Mountain View, Calif.), respectively, and showed greater than 90% purity.

The binding of HIV-2 gp105 and HIV-1 gp120 to $CD4^+$ and $CD8^+$ T cells obtained by these procedures is shown in Fig. 1. Purified $CD4^+$ and $CD8^+$ T cells were incubated with FITC-labeled gp105 and gp120 for 24 h and were analyzed with a FACStar plus (Becton Dickinson) after washing. As shown in Fig. 1A, the binding of gp105 was lower than that of gp120, although both gp105 and gp120 could bind to $CD4^+$ T cells. This may be related to the lower affinity of gp105, which has been described previously (3, 7, 11, 29, 42). The binding of Leu 3a antibody to purified $CD4^+$ T cells was inhibited by pretreatment of cells with gp105 or gp120, and this inhibition was concentration dependent (data not shown). The in vitro binding of recombinant gp120 to $CD4^+$ T cells in this study was similar to that in previous reports (35, 37). However, there have been few reports about the binding of recombinant gp105 to $CD4^+$ T cells in vitro. We next investigated the binding of gp105 and gp120 to $CD8^+$ T cells. As shown in Fig. 1B, gp120 did not bind to purified $CD8^+$ T cells, even at a high concentration. Unexpectedly, gp105 bound to $CD8^+$ T cells in a concentration-dependent fashion (Fig. 1B). This binding to $CD8^+$ T cells was completely inhibited by pretreatment of gp105 (1 μg/ml) with polyclonal anti-gp105 antibodies (0.1 μg/ml) (Intracel Co.) for 1 h (Fig. 2).

To further confirm these observations, we used a variant of MOLT-4 cells obtained by the limiting dilution method, instead of purified $CD8^+$ T cells. This cell line had extremely low CD4 expression and high CD8 expression (0.2 and 83.2% pos-

* Corresponding author. Mailing address: Department of Medicine, Juntendo University Izu-Nagaoka Hospital, 1129 Nagaoka, Izu-Nagaoka-cho, Tagata-gun, Shizuoka 410-22, Japan. Phone: 81-559-48-3111. Fax: 81-559-48-5088.

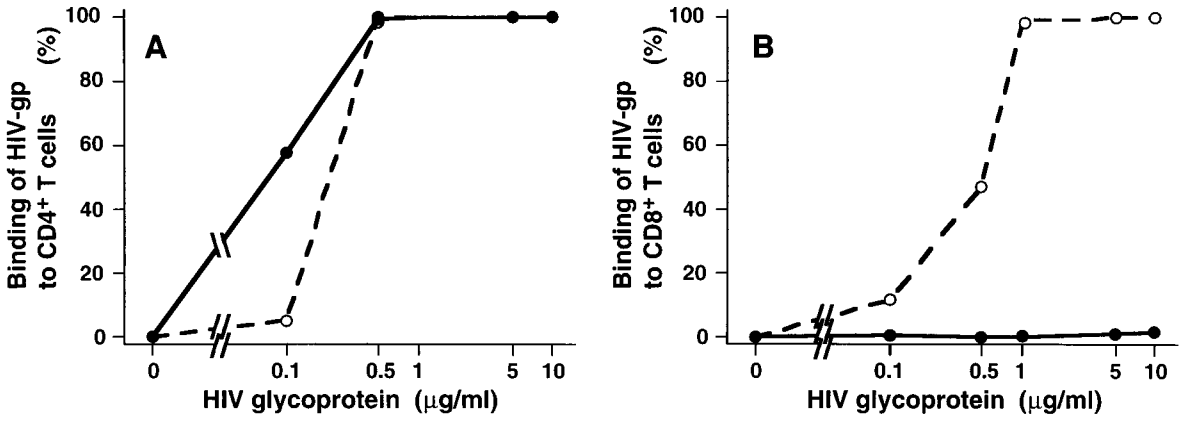

FIG. 1. Binding of HIV-1 gp120 (●) and HIV-2 gp105 (○) to $CD4^+$ (A) and $CD8^+$ (B) T cells purified from PBMC.

itivity for the Leu 3a antibody and the Leu 2a antibody, respectively) compared with parental MOLT-4 cells (70 and 29% positivity, respectively). We confirmed that gp105 showed concentration-dependent binding to this cell line, although gp120 did not bind to it even at a high concentration (Fig. 3).

When we used human CD8 α- and β-chain-expressed mouse T-cell hybridoma (HTB Leu2.18/human CD8β), binding of gp105 (but not of gp120) to this hybridoma was observed (Fig. 4). This hybridoma was established by the transfection of human CD8α cDNA in the expression vector BMG hygro and human CD8β cDNA in the expression vector BMG neo into a mouse T-cell hybridoma (HTB 176.10) (21, 27, 39). These cells were cultured with the medium with 0.15 mg of hygromycin B (Sigma Chemical Co., St. Louis, Mo.)/ml, and positive percentages of human CD8 molecules detected by Leu 2a antibodies were 0.0% in HTB 176.10 and 68.9% in HTB Leu2.18/human CD8β (data not shown). Mouse T-cell hybridomas HTB 176.10 and HTB Leu2.18/human CD8β were cultured with gp105 or gp120 for 24 h at 37°C and were stained with FITC-labeled anti-gp105 antibodies or FITC-labeled anti-gp120 antibodies after washing, and then fluorescence-activated cell sorter (FACS) analysis was performed. The results obtained by this experiment also confirmed that gp105 (but not gp120) can bind to human CD8 molecules (Fig. 4).

For further investigation, we used monoclonal antibodies to CD8 molecules: a Leu 2a antibody (Becton Dickinson), an OKT8 antibody (Ortho Diagnostics, Raritan, N.J.), and an anti-CD8β antibody (Coulter-Immunotech, Westbrook, Maine). CD8 molecules consist of α and β chains. Leu 2a and OKT8 antibodies recognize different epitopes on the α chain of the CD8 molecule (38), while the binding site of the anti-CD8β antibody is located on the β chain and not the α chain of the CD8 molecule.

First, we performed a blocking experiment by gp105 pretreatment. After pretreatment of $CD8^+$ T cells with gp105 for 24 h and washing, the cells were stained with a phycoerythrin (PE)-labeled Leu 2a antibody (Becton Dickinson), a PE-labeled OKT8 antibody (Ortho Diagnostics), or an unlabeled anti-CD8β antibody (mouse immunoglobulin G1), which was detected with a PE-labeled goat anti-mouse immunoglobulin G (Coulter-Immunotech), and FACS analysis was performed to examine the binding of these antibodies. As shown in Fig. 5, gp105 pretreatment strongly inhibited Leu 2a antibody binding and relatively weakly inhibited OKT8 antibody binding to $CD8^+$ T cells. However, anti-CD8β antibody binding to $CD8^+$ T cells was not inhibited by gp105 pretreatment.

When $CD8^+$ T cells were pretreated with an unlabeled Leu

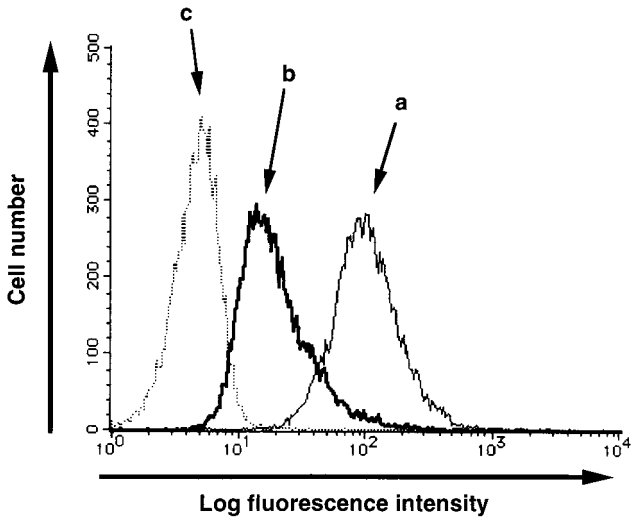

FIG. 2. Blocking effect of anti-gp105 antibodies on the binding of gp105 to $CD8^+$ T cells purified from PBMC. Binding of gp105 (a) was inhibited by pretreatment of gp105 with anti-gp105 antibodies (b). Mean fluorescence intensity was 123.7 (a) or 27.5 (b). c, autofluorescence.

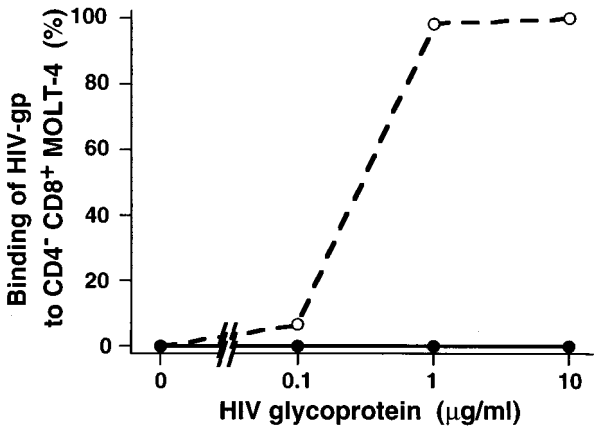

FIG. 3. Binding of gp120 (●) and gp105 (○) to $CD4^-$ $CD8^+$ MOLT-4 cells.

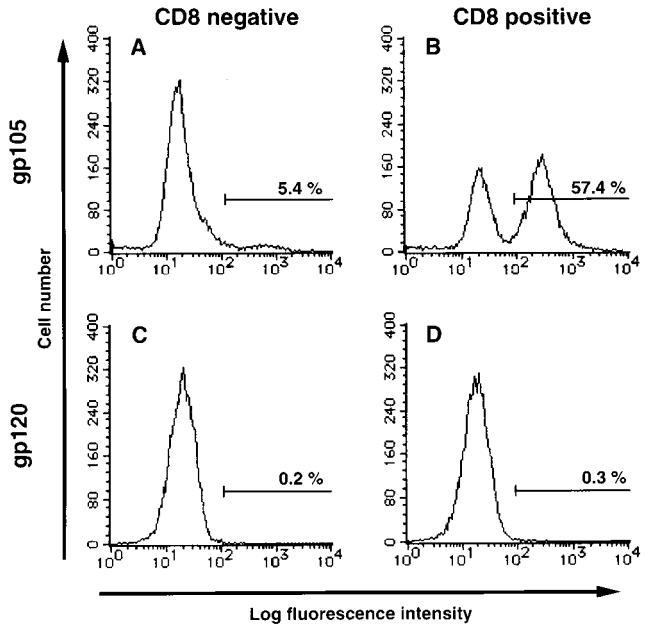

FIG. 4. Binding of gp105 and gp120 to human CD8 molecule-negative (HTB 176.10) and -positive (HTB Leu2.18/human CD8β) mouse T-cell hybridomas. (A) HTB 176.10 cultured with gp105. (B) HTB Leu2.18/human CD8β cultured with gp105. (C) HTB 176.10 cultured with gp120. (D) HTB Leu2.18/human CD8β cultured with gp120. The percentage of binding of antibodies to gp105 or gp120 is shown in each histogram.

2a antibody, an OKT8 antibody, or an anti-CD8β antibody (25 μg/ml) for 2 h at 4°C, the cells were incubated with FITC-labeled gp105 for 24 h after washing, and gp105 binding was assessed by FACS analysis. The concentrations of these three antibodies used for pretreatment of $CD8^+$ T cells were high enough to occupy all the binding epitopes on CD8 molecules that they recognized (data not shown). The binding of gp105 was decreased by pretreatment with Leu 2a antibody (Fig. 6A). This inhibitory effect of Leu 2a antibody was concentration dependent (data not shown). However, gp105 binding was not inhibited by pretreatment with OKT8 antibody or anti-CD8β antibody (Fig. 6B and C). Even when these antibodies were present in high concentrations (100 to 200 μg/ml), they could not block gp105 binding (data not shown). These findings indicated that the binding site of gp105 on CD8 molecules overlapped that of the Leu 2a antibody and that the binding site of the OKT8 antibody on CD8 molecules might partly overlap that of gp105, although the OKT8 antibody did not cover all of the gp105 binding epitope. Furthermore, the binding site of gp105 was not located on the β chain of CD8, recognized by anti-CD8β antibody.

The main receptor for gp105 is thought to be the N-terminal region of CD4, as is the case for gp120, although precise mapping of the gp105 epitope compared with that for gp120 has not been done (7, 33, 34), and Fusin/CXCR4, a member of the chemokine receptor family, has been reported to be able to function as an alternative receptor for some isolates of HIV-2 in the absence of CD4 (15). The interaction between HIV-2 gp105 and its receptor, and the process of fusion of this virus with its target cells, remains unclear. We previously reported that recombinant soluble CD4 (rCD4) can enhance HIV-2 mediated syncytium formation in vitro but can completely inhibit HIV-1-related syncytium formation, although the precise mechanism is still unknown (34). Similar enhancement of syncytium formation by rCD4 was also reported for SIV (1). This suggested that there may be differences in the attachment of virus envelope to CD4 molecules between HIV-1 and HIV-2 or SIV. Although the range of human cells which can be infected by HIV-2 appears to be similar to that for HIV-1, distinct tropism has been reported to exist in vitro for certain laboratory cell lines (9, 10, 18, 31). With regard to SIV, there is evidence that this virus may productively infect $CD8^+$ cells in vitro and in vivo (13, 14, 28, 41). Although binding between the SIV envelope and CD8 remains unclear, these results may be closely related to our findings, and SIV infection may be established via binding of the SIV envelope proteins to CD8 molecules, because the character of SIV is similar to that of HIV-2. Even with regard to HIV-1, its provirus sequences were reported to be detected in $CD8^+$ T cells of HIV-1-infected patients (24). This suggests the existence of unrecognized receptors for this virus. A tropism of HIV for different cell types may be broader than previously described. The differences between the processes of HIV-1 and HIV-2 or SIV infection suggest that there may be fundamental differences in the life cycles of these viruses.

In the present study, we found that HIV-2 envelope glycoprotein was clearly bound to part of the α chain (but not the β chain) of CD8, which was recognized by Leu 2a and OKT8 antibodies. In addition, Leu 2a antibody blocked the critical binding sites of gp105 on the CD8 molecules, but OKT8 antibody did not (Fig. 5 and 6). Because the glycosylation pattern of the envelope glycoprotein could play an important role in the tropism of HIV (22), we cannot rule out the influence of

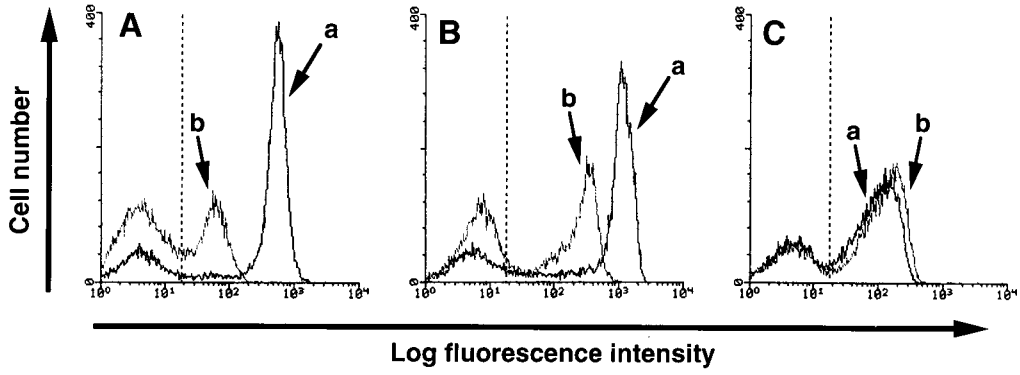

FIG. 5. Blocking effect of gp105 on the binding of anti-CD8 antibodies to $CD8^+$ T cells. The binding of anti-CD8 antibodies Leu 2a (A), OKT8 (B), and anti-CD8β (C) was examined in the absence (a) or presence (b) of gp105 (1 μg/ml). The dashed lines indicate the threshold of autofluorescence in each histogram.

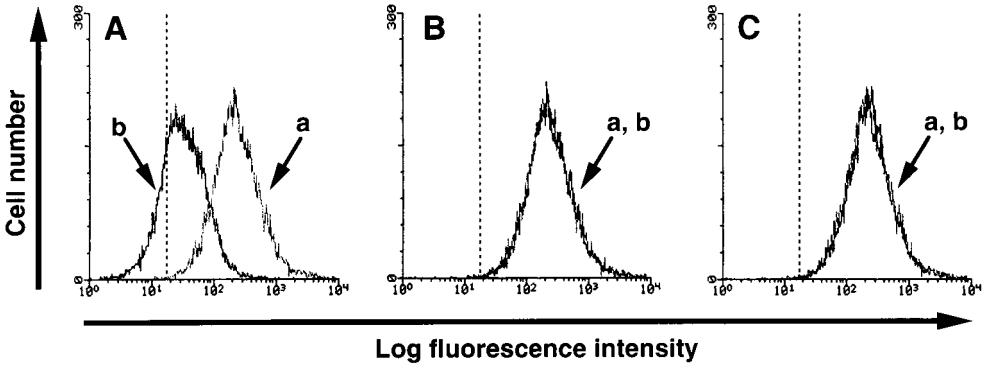

FIG. 6. Blocking effect of anti-CD8 antibodies on the binding of gp105 to $CD8^+$ T cells. The binding of gp105 (1 μg/ml) was examined in the absence (a) or presence (b) of Leu 2a (A), OKT8 (B), and anti-CD8β (C) antibodies. The concentration of each antibody was 25 μg/ml. The dashed lines indicate the threshold of autofluorescence in each histogram. Curve a overlaps curve b in panels B and C.

the glycosylation pattern of recombinant gp105 on binding to $CD8^+$ T cells, although recombinant HIV-1 gp120 obtained by a similar expression system could not bind to CD8 molecules. We are now investigating whether an HIV-2_{ROD} isolate can bind to and infect $CD4^-$ $CD8^+$ cells by using a variant of the MOLT-4 cell line and a mouse T-cell hybridoma transfected with human CD8 molecules (HTB Leu2.18/human CD8β).

Binding of HIV-1 gp120 to CD4 molecules has been reported to induce phosphorylation of CD4-associated tyrosine kinase $p56^{lck}$ in T cells (19). Because the α chain (but not the β chain) of the CD8 molecule is known to be linked to protein tyrosine kinase $p56^{lck}$ (32), HIV-2 gp105 stimulation via the α chain of CD8 may be significant in signal transduction into $CD8^+$ T cells and the resultant production of cytokines and chemokines. The $CD8^+$ T cell and the antiviral factors it releases, including interleukin-16, macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES (regulated upon activation, normal T-cell expressed and secreted), have attracted attention as inhibitors of the replication of HIV (4, 12) and critical factors in preventing disease progression following HIV-1 infection (6, 23). The in vitro production of MIP-1α, MIP-1β, and RANTES derived from gp105-stimulated PBMC was much higher than that from gp120-stimulated PBMC in our culture system (30). This may be related to the prevention of disease progression and the resultant long survival after HIV-2 infection and/or the protection against HIV-1 infection by prior HIV-2 infection which was reported by Travers et al. (40). The differences in production of cytokines and chemokines after stimulation with gp105 or gp120, and the role of gp105-stimulated $CD8^+$ T cells in this production, are being studied further in our laboratory (Juntendo University).

In conclusion, this is the first report stating that the CD8 molecule is one of the receptors for HIV-2. This finding may open up new possibilities for understanding the mechanism of the much slower spread and lower virulence of HIV-2 compared with HIV-1. It may also provide new insight into methods of preventing or delaying the onset of AIDS after HIV-1 infection.

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